

4 BIOLOGICAL SAMPLING

This section provides background material related to biological sampling in headwater streams. The incorporation of biomonitoring (i.e., the use of organisms to evaluate changes in an environment) into assessment programs is advocated because biota are ubiquitous across the landscape, represent a diversity of responses, integrate stressor effects over time, and are relevant to societal needs (Rosenberg and Resh 1993, Whitton and Kelly 1995). The primary biological levels used in biomonitoring are community and population levels, although biological measurements can range from molecules to ecosystems. The response measures in bioassessment are typically abundance, biomass, and diversity; however, there is a trend toward quantifying characteristics (i.e., species traits) and functional roles of biota for predicting biological responses to specific disturbances or stressors. Some of these traits include life span, maximum size, phenology, and physiology (Muotka and Virtanen 1995, Biggs et al. 1998, Charvet et al. 1998, Usseglio-Polatera et al. 2000). Species traits may provide important insight in understanding stressor-specific responses and have a place in bioassessment, as do tolerance values (e.g., Hilsenhoff 1987, Van Dam et al. 1994) and functional feeding groups.

The choice of biological level and group should match the study objectives. Be aware of attributes and limitations of particular taxonomic groups. For example, primary producers will respond immediately to changes in light and nutrients, whereas a lag-response is expected for consumers. Long-term monitoring of individuals is possible for most bryophytes, but not so for invertebrates with relatively short life spans. In addition, ethical and legal considerations (e.g.,

sampling permits) are more prevalent for some biota than others. Particular sampling regimes may also be more conducive to some groups than others. For instance, organisms with a patchy distribution may require larger sample areas (or more samples) than those with a uniform distribution.

As discussed for physical habitat assessments, methods of biological sampling can range from qualitative to quantitative. Sampling methods should match the investigators' study objectives. Objectivity, comparability and precision of the methods increase as the level of quantification increases. For example, quantitative methods measure biota over a specified area or volume (e.g., Hess sampler) with greater precision and repeatability than semi-quantitative (e.g., kick nets) or qualitative methods (e.g., dip net jabs). However, the level of effort (especially time) and training may increase with more quantitative methods. Therefore, when deciding on a sampling method, one should consider the purpose of the resulting data (e.g., species list for the area, statistical comparison among treatments) and the resources available to accomplish the study objectives. Although not discussed in detail in this manual, post-sampling procedures are equally important to consider and should match the study objectives (see Klemm et al. 1990 and Charles et al. 2002).

Their small size and likelihood of drying make headwater intermittent streams unique habitats for sampling biota. Many methods developed for perennial streams may not be as effective or consistent in headwater streams. Water depth and flow may not always be sufficient for some sampling methods. In addition, the sampling area may need to be reduced to

minimize damage to streams and populations (or even to logistically collect a sample). The fluctuation of flow affects the wetted surface area to a greater extent in headwater streams than in larger, downstream water bodies. Therefore, it is critical to monitor wetted surface area when sampling biota. As streams dry, surface water contracts and organisms may track surface water and concentrate (e.g., Stanley et al. 1994). A density increase may be misinterpreted as increased abundance in response to drying if the context of wetted surface area is ignored. Studies that compare biological responses across time periods and/or among habitat types should use sampling methods that are equally efficient across the range of associated hydrologic conditions (Resh 1979, Boulton 1985)

The study objectives and the spatial distribution of the fauna should determine the number of samples or total sample area. Where diversity (or richness) is of interest, species-area relationships should be assessed to determine the appropriate sample area. Under ideal circumstances the number of species collected over area sampled should level-off. Therefore, the appropriate sampling area should coincide with the asymptote (where slope ≈ 0) of the species-area curve. However, because of the diverse and patchy, but numerically skewed nature of aquatic assemblages, the effort needed to reach the asymptote is typically enormous and logistically unattainable (Figure 4-1A). In addition to the preponderance of rare taxa, the limitations associated with fine-scale sample stratification among habitat patches contribute

to the inability to attain the species-area asymptote. An alternative goal that is more feasible to achieve (in time and effort) is the asymptote of the relationship between species gained and sample area (Figure 4-1B). This relationship measures the amount of information gained per unit effort. Similar considerations should be applied where laboratory subsampling is done prior to enumerating and identifying organisms (Vinson and Hawkins 1996, Larsen and Herlihy 1998). Because taxa richness increases with the number of organisms sampled, another consideration when comparing among sites or treatments is to standardize for the number of individuals or rarefy the data (e.g., Downes et al. 1998, McCabe and Gotelli 2000).

The following subsections are organized according to biological group. Included are more traditionally used communities of algae and invertebrates as well as less commonly used bryophytes and amphibians. Sampling methods used in Headwater Intermittent Streams Study are detailed. The identification of indicators of flow permanence was the objective of this study, sampling commonly was restricted to the thalweg. This was done because it was a consistent and conservative target when comparing across sites with varying hydrologic permanence and ecological condition. Other spatial configurations of field samples may be more suitable depending upon the study objectives. Alternative sampling methods are briefly discussed at the end of each subsection.

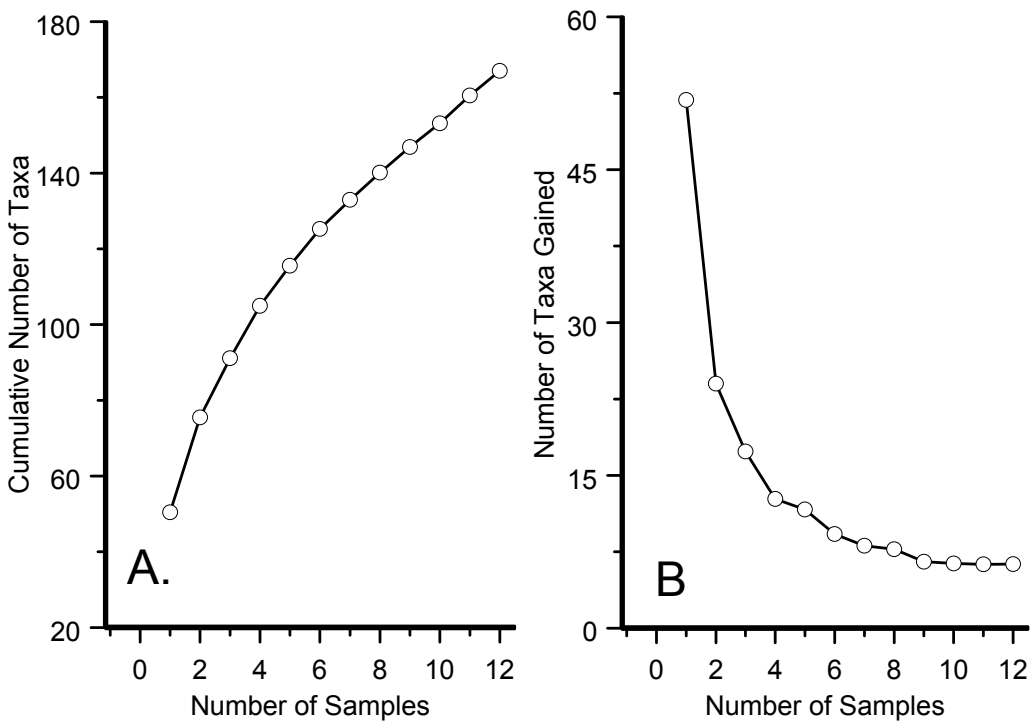


Figure 4-1 Examples of a species-area curve (A) and a species gained-area curve (B) for benthic invertebrates samples (sample area = 0.053 m²) collected from a perennial site on Falling Rock Branch, Robinson Forest, KY. Each point represents the mean (\pm 1 SE) of 100 permutations.

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4.1 Sampling the bryophyte assemblage *General*

This section describes sampling methods for bryophyte assemblages (mosses and liverworts) in headwater streams. Bryophytes include the nonvascular, seedless plants belonging to the classes Musci (mosses, Figure 4-2) and Hepaticae (liverworts, Figure 4-3). Both groups share a life cycle composed of two generations, the sporophyte (spore-producing) and gametophyte (gamete-producing). The sporophyte is directly attached to and nutritionally dependent upon the larger and longer-lived gametophyte.

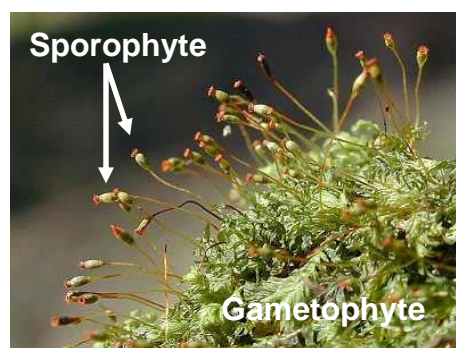


Figure 4-2 Sporophyte and gametophyte generations of a moss. (Photo by Michael Lüth)



Figure 4-3 An epilithic moss (Musci) growing in a headwater stream.



Figure 4-4 An epilithic liverwort (Hepaticae) growing in a headwater stream.

A fundamental gradient that governs the spatial distribution of bryophytes is moisture (Craw 1976, Glime and Vitt 1979). Bryophytes range from being xerophytes (adapted to dry habitats) to obligate hydrophytes (requiring water). This range enables mosses and liverworts to be potentially useful indicators of headwater stream hydrology. Additionally, because bryophytes are sessile and relatively long-lived compared to other stream-dwelling organisms, their distributions may be useful descriptors of hydrologic and ecological conditions over several years. Bryophytes have been used to monitor heavy metals and other pollutants through accumulation in tissue (e.g., Glime 1992, Engleman and McDiffett 1996), biochemical change (Lopez and Carbeilleira 1989), and species composition (Vrhovšek et al. 1984, Stephenson et al. 1995). Shifts in biomass, species dominance and composition of bryophyte assemblages have been linked to changes in water chemistry (Omerod et al. 1987, Bowden et al. 1994), sediment particle size (Vuori and Joensuu 1996), and hydrology (Englund et al. 1997, Downes et al. 2003). Texts for taxonomic identification of bryophytes are referenced at the end of this subsection.

Procedure

Delineate the 30-m study reach so that the measuring tape is positioned along the thalweg.

4.1.1 Qualitative sampling

Bryophyte sampling is confined to the thalweg (deepest flow path) of the 30-m study reach. Avoid unattached specimens or specimens growing on loose woody debris because these may have recently been deposited from adjacent forest or upstream. Most specimens suitable for sampling will be growing on stone substrate and submerged in water (see Figures 4-2 and 4-3). In dry channels be careful to select only specimens growing within the thalweg. If no specimens are found note this on the field form (Figure 4-4). Scrape small samples (~ 10 cm²) of all representative species seen in the thalweg using a scoopula or similar tool. Collect specimens with the sporophyte generation whenever possible, because the sporophyte characteristics are often critical for species-level identification. Place all collected specimens from a study site into a single 24-oz Whirl-Pak® bag with a sample label that includes relevant information (e.g., locality, date, collector's initials). Keep samples cool (cooler with ice bags or ice packs) while transporting them to the laboratory and until the sample can be air-dried in the laboratory. Protect samples from ice meltwater. In the laboratory remove samples and associated labels from Whirl-Pak® bags and place them into paper bags or envelopes for air drying. Write the label information on the outside of the envelopes with a permanent marker.

BRYOPHYTES SAMPLED: Y N	
SAMPLE ID _____	COMMENTS

Figure 4-5 Appropriate location for recording bryophyte sample information on page 1 of field forms.

4.1.2 Quantitative sampling

As opposed to the qualitative sampling described above, quantitative sampling of

bryophyte assemblages requires field identification (or at least recognition of distinct taxa) and therefore some expertise on

the flora (Slack 1984, Bowden et al. 2006). There are three primary measures used to quantify bryophytes by taxa: frequency, percent cover, and standing crop. Frequency measures the proportion of the samples collected that contains a taxon, whereas percent cover measures the proportion of the sample area that is covered by a taxon. Standing crop biomass is a measure of the biomass of a taxon within a sample and is usually reported as g m⁻² dry weight or ash-free dry mass. The advantage of the percent cover and frequency over standing crop biomass is that they are non-destructive, enabling subsequent measurements. Percent cover is usually considered an estimate and therefore more subjective than frequency or standing crop biomass. Some investigators have subdivided the sample area using plexiglass grids or other viewing devices to improve repeatability. In addition, rather than using absolute percentages, percentage categories are often used when estimating cover (e.g., Braun-Blanquet cover scale, see Mueller-Dombois and Ellenberg 1974, Bowden et al. 2006). Voucher specimens for each taxon are collected for later identification or confirmation in the laboratory.

Three sampling methods commonly used and are listed by increasing level of effort: point-intercept, transect, and quadrat (i.e., plot). Points, transects and quadrats should be randomly or haphazardly selected across the study reach to avoid samples biasing their reach representation. Depending upon the study objectives, it may be useful to stratify sampling within a study reach (e.g., habitat type, stream margin versus mid-channel, height relative to water surface). Stratified sampling (or floristic habitat sampling) has been advocated when compiling comprehensive surveys of bryophyte diversity (Newmaster et al. 2005). The point-intercept method uses a grid or coordinate system.

Each randomly or haphazardly selected coordinate (point) is sampled by simply recording the species present at the point, on the nearest substrate (e.g., cobble), or a surrounding area (making it similar to quadrat sampling). The transect method uses randomly or haphazardly placed transects (measuring tape or string) typically positioned perpendicular to the direction of flow. Sampling along transects may span only the wetted width, entire active, or into the adjacent riparian zone. Percent cover for each species is determined by the percent of the transect length that is intercepted by each species. Frequency may be assessed among transects or within transects by recording individual species-patches along each transect. Some investigators treat transects as belts, where bryophytes are sampled within a set distance (e.g., 0.1 m) upstream and downstream of each transect (e.g., Steinman and Boston 1993, Suren and Duncan 1999). The quadrat method uses circular, square, or rectangular plots of known area that are randomly or haphazardly positioned in the study area. The percent cover of each species within the quadrat is recorded and frequency is typically assessed across replicate quadrats. Some investigators have sampled quadrats along transects to quantify assemblage shifts across geomorphic units (e.g., Jonsson 1996). Potential edge effects (perimeter:area) are lower for circular plots than for square or rectangular plots (Krebs 1999). The number and size of replicate sampling units depends on the patchiness of bryophytes with the study reaches and the resources available for the study. Studies comparing transect, point intercept, and quadrat methods have generally found similar estimates of bryophyte and macroalgae abundance (Rout and Gaur 1990, Neechi et al. 1995). However, because the quadrat method usually covers a larger sampling area, this method will include more

rare taxa and tend to have higher estimates of taxa richness.

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- Equipment and supplies*
 Measuring tape (50 m)
 Metal scoopula or spatula
 24 oz Whirl-Pak® bags
 Pencils
 Permanent marker
 Label paper
 Field forms
 Cooler
 Ice or ice packs
 Paper bags or envelopes
- 4.2 Sampling the epilithic algal assemblage**
General
 This subsection describes methods for sampling the algal assemblage in headwater streams. The particular algae sampled in these procedures are epilithic algae (or algae associated with stone surfaces) and includes diatoms (Bacillariophyta) and “soft” algae (i.e., Chlorophyta, Cyanophyta, Rhodophyta, and Chrysophyta). Epilithic algae are associated with fungi, bacteria, heterotrophic protists, and organic matter and together they form a matrix called periphyton, biofilm, or aufwuchs. The target organisms for laboratory identification are the algae within the periphyton, but because algae are difficult to exclusively collect, the periphyton is sampled. Algal assemblages have been shown to be useful indicators of ecological condition in wadable streams (e.g., Pan et al. 1996, Hill

et al. 2000). The ubiquity, diversity, sampling efficiency, and responsiveness to physical and chemical stressors are all attributes for the use of algae in bioassessment (Patrick 1973, Stevenson and Lowe 1986). Despite being ubiquitous, algae have received less attention than invertebrates in temporary streams research (see review by Stanley et al. 2004). Algae are potentially useful indicators of hydrologic permanence because algae inhabit a wide range of habitats (terrestrial to aquatic) and varying in desiccation tolerance and presence of resistant structures (e.g., akinetes, cysts, zygotes, mucilage) among taxonomic groups (Davis 1972).

4.2.1. Quantitative sampling of epilithic algae

This first procedure is modified from the procedure described by Hill (1998) and focuses only on the collection of periphyton on natural substrates to determine the taxonomic composition of the algal assemblage (by abundance and biovolume). The algae assemblage is sampled during each season (spring and summer) because it is likely to vary with season.

Procedure

Delineate the 30-m study reach so that the measuring tape is positioned along the thalweg. Identify erosional and depositional habitats with the study reach. Two separate algae samples are taken from each study reach, one from each habitat type (erosional and depositional). Sampling is confined to the

thalweg of the study reach and is done regardless of hydrologic condition. Each sample is a composite of 12 cm² areas from upper surface of 6 individual stones.

4.2.1.1. Substrate collection

Begin by haphazardly collecting 6 stones (>12 cm² upper surface area) from the thalweg of a habitat type and placing them in the large basin with the upper surface facing upward. Avoid disturbing the streambed as much as possible when collecting stones and make sure that the stones have not been disturbed by other sampling activities (communicate with fellow crewmembers). Spread the sampling across multiple units of each habitat type along the study reach. However, where hydrologic conditions vary among units of (or stones from) a habitat type in a study reach (i.e., there are pools with and without surface water), restrict sampling to the dominant hydrologic condition represented by the habitat units within the study reach. For example, if a study reach has 5 depositional habitat units and 4 had surface water and one was dry, collect the 6 stones from the 4 wet units. Indicate on the field form the number of stones collected and whether the stones collected were wet or dry (Figure 4-5). Stones can be randomly selected within available habitat units in the reach. Numbers ranging from 1 to 100 can be drawn and the nearest stone along the thalweg coinciding proportionally with the unit length is selected.

ALGAE				
# STONES SAMPLED: Depositional Habitats <u>6</u> Erosional Habitats <u>6</u> TOTAL AREA SAMPLED: <u>144</u> cm ² (each 12 cm ²)				
SAMPLE ID	HABITAT TYPE	NUMBER OF BAGS	COLLECTED BY:	COMMENTS
Four – JC – 1 - D	Depositional	1 of 1	KMF	Wet
Four – JC – 1 - E	Erosional	1 of 1	KMF	Wet

Figure 4-6 Appropriate location for recording algal sample information on page 1 of field forms. Example information is shown in red.

4.2.1.2. Compositing and preserving sample
Fill the wash bottle to the 50-ml mark with stream water. Place a 1.5" diameter PVC circle (i.e., delimiter) on the upper surface of a stone to define a 12 cm² area. Use the metal spatula and a firm-bristle toothbrush (trim bristles to half original length) to dislodge algae from the stone surface within the delimiter (Figure 4-6). Rigorously scrape and brush the surface for 30 seconds. Be aware that because clay particles have similar density (mass per unit volume), excess clay particles in the sample may hamper identification and enumeration of algae. Using the wash bottle, sparingly wash the dislodged algae from the delimited area into the small plastic container. Repeat this step with the 5 remaining stones to make a composite sample. Use the remaining water in the wash bottle to rinse off tooth brush and spatula into the small basin. Pour the composite sample from the small container through the small funnel and into a 50-ml centrifuge tube. Use a syringe or bulb pipette to add 2 ml of 10% formalin to preserve the sample*.

If formalin is not taken to the field, keep sample in the dark and on ice until it is preserved. Tightly cap the centrifuge tube and seal with electrical tape. Gently shake the tube to distribute the formalin throughout the sample. Make a label (waterproof paper and pencil) that includes relevant information (e.g., locality, habitat type, date, collector's initials). Attach the sample label securely to the outside of the centrifuge tube using packing tape or clear tape strips. Also write

* Wear gloves and safety eyeglasses when using formalin and work in a well-ventilated area. Formalin is extremely caustic and potentially carcinogenic. It may cause severe irritation on contact with skin and eyes. Rinse immediately with water in case of contact with skin or eyes.

label information on the field form (Figure 4-5). Thoroughly rinse all sampling equipment with clean stream water to prevent cross-contamination between samples. If additional measures of biomass and/or pigment are needed, the sample can be split volumetrically (see Hill 1998).

Repeat procedures outlined in 4.2.1.1 and 4.2.1.2 for the remaining habitat type.



Figure 4-7 Collecting epilithic algae from a stone within the sample delimiter.

4.2.1.3. Sample transport and shipping
Before leaving a site, check that all samples are labeled properly and safely stowed. At the vehicle, consolidate all algae samples in one location. All the samples can then be placed in a crush-resistant and leak-proof container and transported to the laboratory for processing. If samples need to be shipped, include any special shipping forms that may be required for the formalin-preserved samples.

4.2.2 Alternative methods

Here we briefly describe various techniques used to sample epilithic algae in streams. More details are available in reviews by Stevenson and Lowe (1986) and Aloï (1990). Algal sampling methods can be separated into

two broad categories: natural substrates and artificial substrates. As previously described, natural substrate sampling involves quantitatively collecting epilithon found growing naturally on substrates in streams. In contrast, artificial substrate sampling involves placing substrates (e.g., glass slides, ceramic tiles, bricks) into streams for periphyton colonization. Because the exposure time is known, artificial substrates provide the investigator with more control and perhaps less variability among sampling units than natural substrates. However, assemblages colonizing artificial substrates may not provide a realistic characterization of the algal assemblage in streams. Taxonomic composition and measures of biomass (chlorophyll *a* and AFDM) on artificial substrates can differ from these algal measures on natural substrates (Lay and Ward 1987, Cattaneo and Amireault 1992). Homogeneous substrate texture and short incubation times of artificial substrate procedures have been identified as the likely causes for lower algal biomass and lower representation by green and blue-green algae than seen in adjacent natural substrates. Both methods have benefits and drawbacks for monitoring algal assemblages and these should be weighed carefully when designing monitoring studies.

Algae in headwater streams are often logistically easier to collect than from deeper rivers and lakes. Many substrate types are easily removed from the stream for subsequent collection of algae. Rather than subsampling periphyton on a substrate particle, several investigators have used the entire substrate as a sampling unit (e.g., Biggs and Close 1989, Dodds et al. 1999, Mosisch 2001). Surface area of substrates can be estimated using substrate dimensions and geometric equations (e.g., Graham et al. 1988). Others have determined stone surface area by covering stones with aluminum foil,

plastic wrap, or ink stamps (Doeg and Lake 1981, Lay and Ward 1987). Surface area – weight relationships are then used to determine surface area of substrates. Large boulders and bedrock common to steep headwater streams can pose a problem in retrieving samples and quantifying surface area. Syringe type samplers (e.g., Loeb 1981, Flower 1981, Peters et al. 2005) offer a solution, where a sample can be collected *in situ*. Syringe samplers use brushes to remove attached periphyton within an enclosed area; then the sample is suctioned and transferred onto a filter or into a sample container. One drawback noted about syringe samplers is an underestimation of chlorophyll *a* concentrations from stream samples but not from lake samples (Cattaneo and Roberge 1991). Sampler brushes are likely ineffective at removing tightly attached members of the periphyton assemblage in streams. Davies and Gee (1993) developed a scouring disc for periphyton removal and reported higher concentrations of chlorophyll *a* were obtained from the scouring disc than from either brushing or scraping. Algae associated with fine particles (epipsammon and epipelon) can be sampled simply by using an area delimiter (e.g., inverted petri dish) and spatula or trowel. The delimiter is positioned into the upper sediment layers and the spatula is positioned beneath. The spatula is carefully lifted and the sample is transferred to a sample container using a funnel. Because algae may be firmly attached to sand grains, additional laboratory steps (i.e., sonication) are needed prior to microscopic or fluorometric measurement (Miller et al. 1987, Romaní and Sabater 2001). Further details on sampling algae from various substrates are discussed in Moulton et al. 2002.

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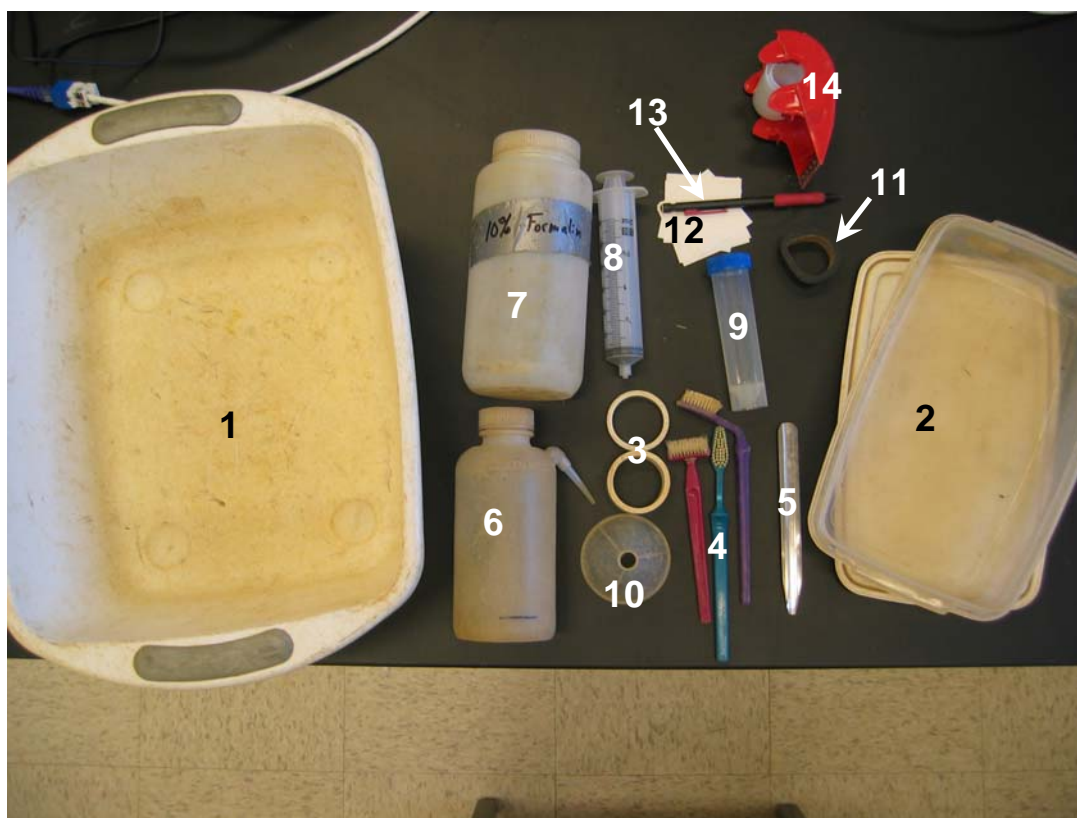


Figure 4-8 Equipment used to collect and preserve algal assemblage samples. Numbers correspond to the Equipment and Supplies list.

Equipment and supplies (Numbers correspond to items in Figure 4-7)

Measuring tape (50 m)

1. Plastic wash basin (approximately 35 cm x 29 cm x 14 cm)

2. Small plastic container or basin (e.g., Tupperware® or Rubbermaid® container) (approximately 25 cm x 16 cm x 6 cm, large enough to contain a cobble and large gravel particle and can be used to store items listed below)

3. PVC ring delimiter (1.5 in or 3.8 cm diameter pipe cut 2 to 3 cm in length)

4. Firm-bristle toothbrush (2) – trim bristles to half their original length

5. Spatula or scoopula

6. Water squirt bottle (with 50 ml volume marked)

7. Buffered formalin (10%)

8. Small syringe or bulb pipette

9. 50 ml centrifuge tubes

10. Small funnel

11. Electric tape

12. Label paper

13. Pencils

14. Packing tape or clear tape strips

4.3 Visual and tactile assessment of algal cover

General

This subsection provides instructions for rapidly assessing algal cover in headwater streams. The method uses a categorical index, Algal Cover Index (ACI) that is based on visual and tactile characteristics of periphyton (and associated algae). The ACI scores and associated characteristics are shown in Table

1. The ACI has been field tested and ACI scores have explained 68-85% of the variation in measured levels of algal (chlorophyll a) and periphyton (AFDM) biomass in streams (Feminella and Hawkins 2000). The protocol described here is modified from Hawkins et al. (2001). Another field-based rapid periphyton method that separately characterizes macroalgal and microalgal cover is described in Stevenson and Bahls (1999).

EMAP protocols include percent classes for filamentous algae (Kaufmann and Robison 1998). The NAQWA qualitative algae sampling protocol includes designating the abundance classes (dense to none) for periphyton at a site (Moulton et al. 2002). The ACI is measured during each season (spring and summer) because it is likely to vary with season.

Table 4-1 Algal Cover Index (ACI) scores and their associated characteristics

ACI Score	Visual and Tactile Characteristics
1	substrate is rough with no apparent growth
1.5	substrate is slimy, but biofilm not visible (i.e., tracks from scratching rock with back of fingernail is not visible)
2	thin layer visible (0.5-1 mm thick, i.e., tracks from scratching rock with back of fingernail is visible)
3	algal mat thickness ranges from 1-5 mm thick and filamentous algae is rare
4	algal mat thickness ranges from 5-20 mm thick and filamentous algae common
5	algal mat thickness >2 cm and/or filamentous algae dominates

Procedure

Delineate the 30-m study reach so that the measuring tape is positioned along the thalweg. Algal cover assessment is based on ≥ 25 substrate particles on the streambed surface. Particles assessed should be spread along the thalweg of the entire study reach. Final ACI score for the study reach is based on the dominant score of the assessed particles. Where there is a clear discrepancy between habitat types (i.e., depositional and

erosional), note ACI scores for both habitat types. Algal cover can be assessed while sampling benthic invertebrates (assessing surface cobble and gravel while scrubbing attached invertebrates). Photographic examples of ACI scores are shown in Figure 4-8. The ACI score is circled on the field form (Figure 4-9). The ACI scores and associated characteristics are also listed on the bottom of page 3 of the field forms.

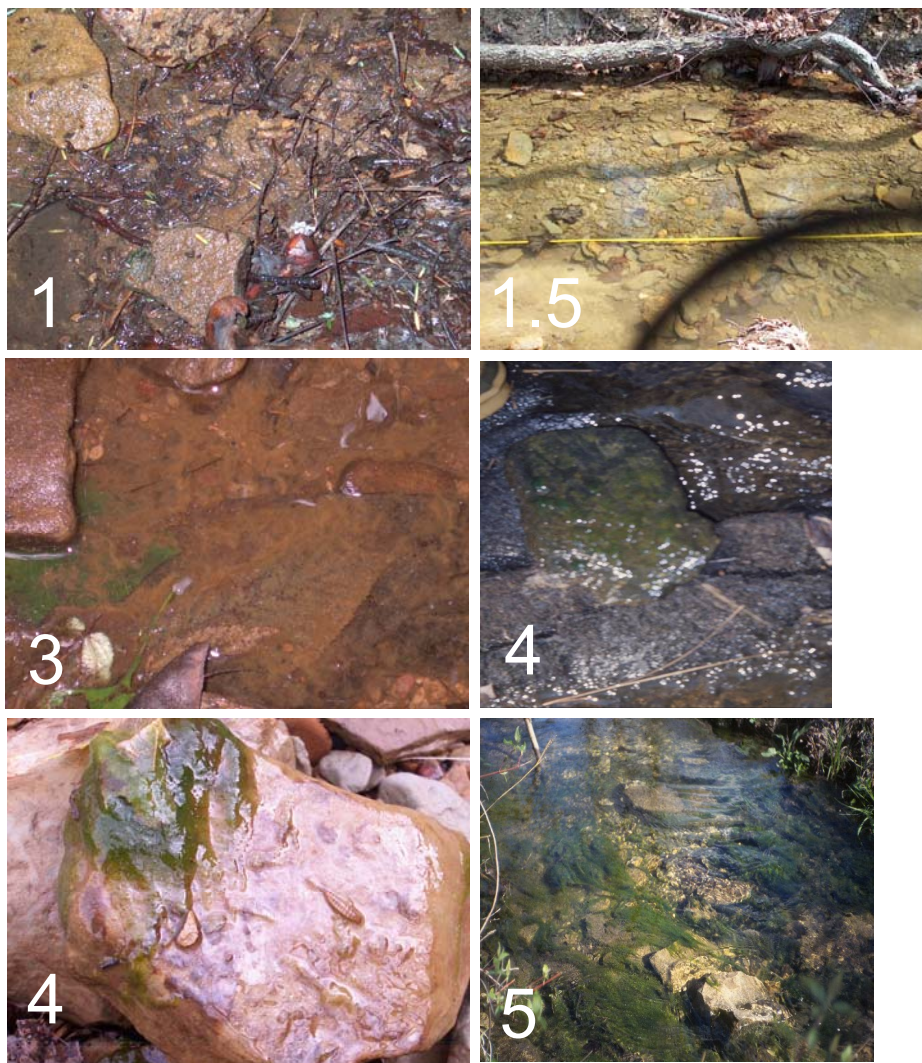


Figure 4-9 Categorical examples of algal cover based on visual and tactile characteristics. Numbers represent Algal Cover Index (ACI) scores associated with periphyton on stones in the photographs.

PRESENCE OF HEADCUT IN REACH		ALGAL COVER INDEX						# CORES FOR SUBSTRATE MOISTURE (depositional)
Y	N	1	1 ^{1/2}	2	3	4	5	

Figure 4-10 Appropriate location for recording the dominant reach score for the Algal Cover Index on page 1 of field forms.

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Equipment and supplies

Measuring tape (50 m)

Field forms

4.4 Sampling the benthic invertebrate assemblage

General

This subsection provides methods for quantitatively sampling the benthic invertebrate assemblage in headwater streams. Benthic invertebrate surveys are widely used to evaluate the condition or health of water bodies (Hellawell 1986, Rosenberg and Resh 1993, Rader et al. 2001). Invertebrate assemblages are composed of a wide range of taxonomic and functional groups, many of which can be found in headwater streams. Furthermore, a diversity of life histories (e.g., voltinism, cohort production interval, dormancy stages) and physiological tolerances are found among aquatic invertebrates (Williams 1996, Frouz et al. 2003). Habitat characteristics (e.g., predictability, disturbance intensity, productivity) set the template governing the evolution of life histories and therefore the composition of assemblages (Southwood 1977, Townsend and Hildrew 1994). Flow is considered one of the ultimate drivers of lotic systems (Lytle and Poff 2004), and may be even more critical to temporary water bodies (Walker et al. 1995, Schwartz and Jenkins 2000). Thus, the composition of invertebrate assemblages should reflect the flow permanence in headwater streams. However, among past investigations there is no consensus regarding the distinctiveness of invertebrate communities among stream reaches of different flow permanence (Deluchi 1988, Feminella 1996, Dietrich and Anderson 2000, Fritz and Dodds 2002, Price et al. 2004). As is often the case in ecological systems, this disparity suggests that the relationship between flow permanence and assemblage organization may be complex.

Plasticity of life histories, subtle variation of drying intensity, degree of connectivity to refugia, physiographic variation, anthropogenic impacts, and other factors may influence assemblage structure.

4.4.1 Quantitative bucket sampling for invertebrate assemblage

The surface water conditions of many headwater streams fluctuate from continuous flow to only standing water in pools to complete absence. Therefore, the method used to collect benthic invertebrates needs to be effective and consistent across the range of hydrological conditions seen in headwater streams. Many existing sampling methods take advantage of flowing conditions to trap invertebrates in nets positioned downstream of the sampling area (e.g., Surber sampler, kick net); however, flow in headwater streams is often too low (sometimes absent) to effectively use these methods. The quantitative bucket sampling method described here is: 1) not dependent upon flow conditions, 2) performed by a single operator, 3) light weight, and 4) inexpensive. The bucket sampling method is modified from methods described by Wilding (1940) and Statzner (1981). The sample area of a 5 gallon bucket sampler is 0.053 m^2 (26-cm diameter). A smaller sample area (e.g., coffee can) may be required to collect benthos from step-pool streams dominated by boulders and large woody debris. The benthic invertebrate assemblage is sampled during each season (spring and summer) because it is likely to vary with season.

Procedure

Before hiking to the study reach(es) make sure all the equipment is stowed in the backpacks and there are ample Whirl-pak bags and ethanol. Transfer the 95% ethanol (usually from 5 gal. container) into transport jug(s) using a funnel. Typically 1 to 1.5 liters is

sufficient to preserve all the samples taken from a study reach.

Delineate the 30-m study reach so that the measuring tape is positioned along the thalweg. Identify erosional and depositional habitats within the study reach (see Subsection 3.4 for designating habitat units). Preliminary data from perennial and intermittent headwater streams in Kentucky, Indiana, and Ohio indicate that the number of additional taxa collected within a 30-m reaches an asymptote after 8 samples (0.42 m^2 , see Figure 4.1B). We recommend that investigators independently assess species-area curves for study reaches, particularly if estimation of invertebrate diversity (a common metric used in ecological condition assessments) is an objective.

Eight separate invertebrate samples (each 0.053 m^2) are haphazardly taken from each study reach, 4 from each habitat type (erosional and depositional). Samples are kept separate to 1) determine the sampling effort needed to sufficiently represent the invertebrate assemblage and 2) provide within-reach measures of variance. Where these objectives are not a concern, samples may be composited. Sampling is confined to the thalweg of the study reach and only where surface water is present (see Subsection 3.1 for designating hydrologic condition). For instance, if there is continuous surface flow throughout the study reach, 4 samples are taken from each habitat type. If there is surface water only in depositional units, only 4 depositional samples are taken. Samples should be spread across multiple units of each habitat type (i.e., erosional and depositional) along the study reach. Where the study reach has ≤ 1 habitat unit (e.g., a pool) with surface water and the other units of that type are dry, do not sample that habitat type. Do not sample areas where the water depth exceeds

the height of the bucket sampler. Samples can be randomly positioned within available habitat units in the reach. For example, numbers ranging from 1 to 100 can be drawn and the center of the bucket sampler is positioned along the thalweg coinciding proportionally along the unit length.

4.4.1.1. Sample collection and preservation
Attach the canvas skirt to the bucket sampler by sliding the elastic band of the canvas skirt over and around the bottom edge of the bucket sampler (Figure 4-11). A foam ring, rather than the canvas skirt, should be fitted to the bucket where the streambed is bedrock. Sample collection should proceed in an

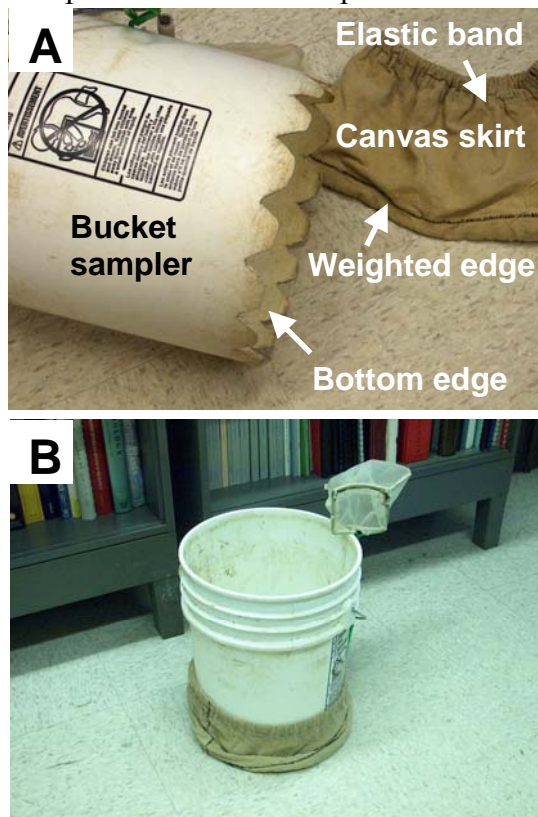


Figure 4-11 Photographs of bucket sampler and canvas skirt A) unassembled and B) assembled for sampling.
upstream direction. Avoid disturbing the streambed outside of the sampling area as much as possible by walking along the banks rather than in the thalweg. Make sure that the

areas sampled have not been disturbed by other sampling activities. Begin by identifying a sampling location within the channel thalweg and place the wash basin, sieve, hand net, and trowel on a bank or gravel bar near the sampling location. With both hands lift the weighted edge of the canvas skirt above the bottom edge of the sampling bucket (to prevent the skirt from being inside the sampling area once the bucket encloses the sampling area). Push the bottom edge of the bucket 3 to 5 cm vertically into the streambed. Adjust the weighted edge of the skirt to seal the sampling area. By hand remove the coarse surface substrates (i.e., large gravel and cobble) from the enclosed sampling area and place them into the wash basin or sieve to be scrubbed (Figure 4-12). Stir by hand or trowel the remaining substrate within the bucket for 10 seconds to a depth of 10 cm (or bedrock, whichever is shallower). This step helps to suspend invertebrates from streambed interstices into the water column.



Figure 4-12 Coarse surface substrate set aside in basin for scrubbing.

Immediately sweep the hand net through the water column for 10 seconds to capture the suspended invertebrates (Figures 4-12 and 4-13). Repeat the substrate stirring and net sweeping steps 2 more times. After the sweeping is completed, empty the net contents into the wash basin. Look for any



Figure 4-13 Sweep the hand net through the water column to collect suspended invertebrates within the bucket area.

invertebrates that may be attached to the net and put these in the basin or sieve. (If the net becomes full before completing the three sets of sweepings, empty its contents into the basin and then continue sampling.) Where the water depth within the bucket area is too shallow to effectively sweep the water column, additional substrate will need to be excavated and placed into the wash basin.

Remove all invertebrates attached to coarse surface substrate by scrubbing them by hand or scrub brush into the sieve or basin (Figure 4-14). Carefully add stream water to the basin (Figure 4-15). Rinse invertebrates from large detritus (e.g., leaves and sticks) that may be in



Figure 4-14 Scrubbing attached invertebrates off the coarse surface substrate in the wash basin (or sieve).



Figure 4-15 Carefully adding water to the wash basin before sample elutriation.

the basin and discard the detritus. Elutriate the remaining contents by swirling the basin by hand and pouring the water and low-density contents (e.g., invertebrates and fine organic matter) into the sieve (Figure 4-16). This step will separate most of the fine sediment particles from the invertebrates. Repeat the elutriation until no organic matter is remaining in the basin. Carefully search the remaining basin contents for heavy-bodied invertebrates (i.e., mollusks, mineral-cased caddisflies, Figure 4-17). Place any heavy-bodied invertebrates into the sieve. Empty



Figure 4-16 Sample elutriation in the wash basin and pouring invertebrates and fine detritus into the sieve.



Figure 4-17 Carefully search the basin for heavy-bodied invertebrates that were not transferred to the sieve.

and rinse the wash basin. At the stream edge carefully wash the sieve contents to one side by gently agitating the sieve while the sieve mesh is partially submerged in water (Figure 4-18). With the wash basin positioned underneath, transfer the majority of the sieve contents by hand or a minimal amount of water using a wash bottle into a 24-oz Whirl-Pak® bag or other container. Agitate the sieve again if necessary to combine the remaining contents against one side of the sieve (Figure 4-19). Wash the remaining sieve contents into the bag with 95% ethanol using the wash bottle (Figure 4-20). Ensure



Figure 4-18 Washing sieve contents to one side by gentle agitation while sieve is partially submerged.



Figure 4-19 Sieve contents condensed for transfer to sample bag.

there is enough space in the bag to sufficiently preserve the sample with additional ethanol. Use more than one bag if necessary to contain a sample. Pour more ethanol into the bag until the sample is completely submerged and the final preservative concentration is $\geq 70\%$ ethanol. Note that the amount of ethanol needed for sufficient preservation increases with the amount of organic matter within a sample.



Figure 4-20 Sieve contents rinsed into sample bag (over basin) using ethanol squirt bottle.

4.4.1.2. Sample labeling

Make a label (waterproof paper and pencil) that includes relevant information (e.g., locality, habitat type, date, collector's initials). Where more than one bag is needed to contain

an entire sample, indicate this on the label by writing “1 of 2”, “2 of 2”, etc. Place label inside the Whirl-Pak ® with the sample and seal the bag. When sealing the bag, remove as much air space as possible from the bag (this will make samples more compact for transport). Seal the bag by folding the tab

over a couple of times then while holding the wire ends, whirl the bag 3-4 times, and lastly twist wire ends together. Write label information on the field form (Figure 4-21) and on the outside of the bag with a permanent marker.

INVERTEBRATES				
# BUCKET SAMPLES: Depositional Habitats <u>4</u> Erosional Habitats <u>4</u> TOTAL AREA SAMPLED: <u>0.4</u> m ² (each ~0.05 m ²)				
SAMPLE ID	HABITAT TYPE	NUMBER OF BAGS	COLLECTED BY:	COMMENTS
Four – JC – 1 – D (1-4)	Depositional	5	KMF	Rep #1 in 2 bags
Four – JC – 1 – E (1-4)	Erosional	6	KMF	Reps. 3 & 4 in 2 bags

Figure 4-21 Appropriate location for recording invertebrate sampling information on page 1 of field forms. Example sample information shown in red.

Repeat procedures outlined in 4.4.1.1 and 4.4.1.2 for the remaining sample replicates in each habitat type. Thoroughly rinse sampling equipment with stream water between sample replicates to prevent transporting any attached invertebrates.

4.4.1.3. Sample transport and shipping

Before leaving a site, check that all samples are labeled properly and safely stowed in a backpack or container (e.g., 5-gallon bucket) for transport to the vehicle. At the vehicle consolidate all invertebrate samples in a crush-resistant and leak-proof container (e.g., cooler) for transport to the laboratory for processing. If samples need to be shipped, include any special shipping forms that may be required for the ethanol-preserved samples.

4.4.2 Alternative methods

Here we will briefly identify other flow-independent methods for quantitatively

sampling macroinvertebrates. More detailed reviews of sampling methods for stream invertebrates can be found in Peckarsky (1984), Klemm et al. 1990, and Merritt et al. (1993). Among the simplest methods is stone sampling, where individual stones are used as the sampling units (Dall 1979, Doeg and Lake 1981, Wrona et al. 1986, Scrimgeour et al. 1993). Stone surface area is estimated as described in Subsection 4.2.2. Some advantages of this method include: 1) simplification of streambed heterogeneity, 2) represent natural sampling units, and 3) efficient, cost-effective method (samples contain little detritus from which to sort invertebrates). For small headwater streams this method also causes minimal degradation to the habitat (if stone area or dimensions are measured immediately after collection) and logistically feasible where channel width limits use of larger samplers. Some disadvantages of stone sampling are the

exclusion of some habitats (e.g., interstitial spaces, bedrock), overestimation of extrapolations to 1 m², and depending upon the typical stone surface area, this method may require large sample sizes to reduce sample variability (Morin 1985).

Like the bucket sampler, vacuum samplers (Boulton 1985, Brown et al. 1987, Brooks 1994) are flow-independent and can be used with equal efficiency across habitat types and flow conditions. Vacuum samplers are devices that enclose a sampling area and transfer sample material by bilge or peristaltic pump to a sieve or net. The major drawbacks to vacuum samplers are related to their overall size. Most of these samplers require more than one person for sample collection and are likely too heavy or unwieldy for long hikes often necessary to reach headwater study sites.

Artificial substrates, such as rock baskets and multi-plate samplers, are commonly used for comparing invertebrate assemblages among sites (e.g., Poulton et al. 2003, Rinella and Feminella 2005). Artificial substrate methods provide the investigator with control over the colonization or exposure time and the standardized size may reduce sample variability. Artificial substrates minimize streambed disturbance to small headwater streams. Some limitations of artificial substrates are differential colonization among taxa, the requirement of multiple visits for deployment and retrieval, and susceptibility to vandalism or natural disturbance.

Modifications to standardized substrates (e.g., Hester-Dendy multiplate samples) may be required to ensure complete submergence in shallow headwater streams (e.g., Winterbourn 1982). Variable submergence among units counters one of the advantages of artificial samplers.

Aquatic insects often represent a significant proportion of the invertebrate assemblage in headwater streams. A wide variety of traps have been used to capture the adult life stage of aquatic insects as they emerge from streams (Davies 1984). Emergence traps can be designed to collect and preserve emerging adults daily or for up to several weeks (LeSage and Harrison 1979, Whiles and Goldowitz 2001). This method can be used to easily sample across a wide range of hydrologic permanence because it is not dependent upon the presence of water (Progar and Moldenke 2002, Price et al. 2003). However, this method requires multiple visits, is susceptible to vandalism or natural disturbance, excludes invertebrate taxa that do not have a winged-adult stage and, depending upon trap design and position, may differentially capture taxa.

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- Equipment and supplies (Numbers correspond to items in Figure 4-22)*
- Measuring tape (50 m)
 1. Bucket sampler = 5-gallon bucket with bottom cut out w/ bottom area $\sim 0.053 \text{ m}^2$
 2. Canvas skirt
 3. Hand-net (243 μm mesh)
 4. Plastic wash basins
 5. Hand trowel
 6. 250 μm sieves
 7. Funnel
 8. Ethanol transport jug(s)
 9. Squir bottle (250 ml)
 10. 24 oz Whirl-pak bags or other sample containers
 11. Label paper
- Ethanol (95%)
Sharpies & Pencils
Field forms

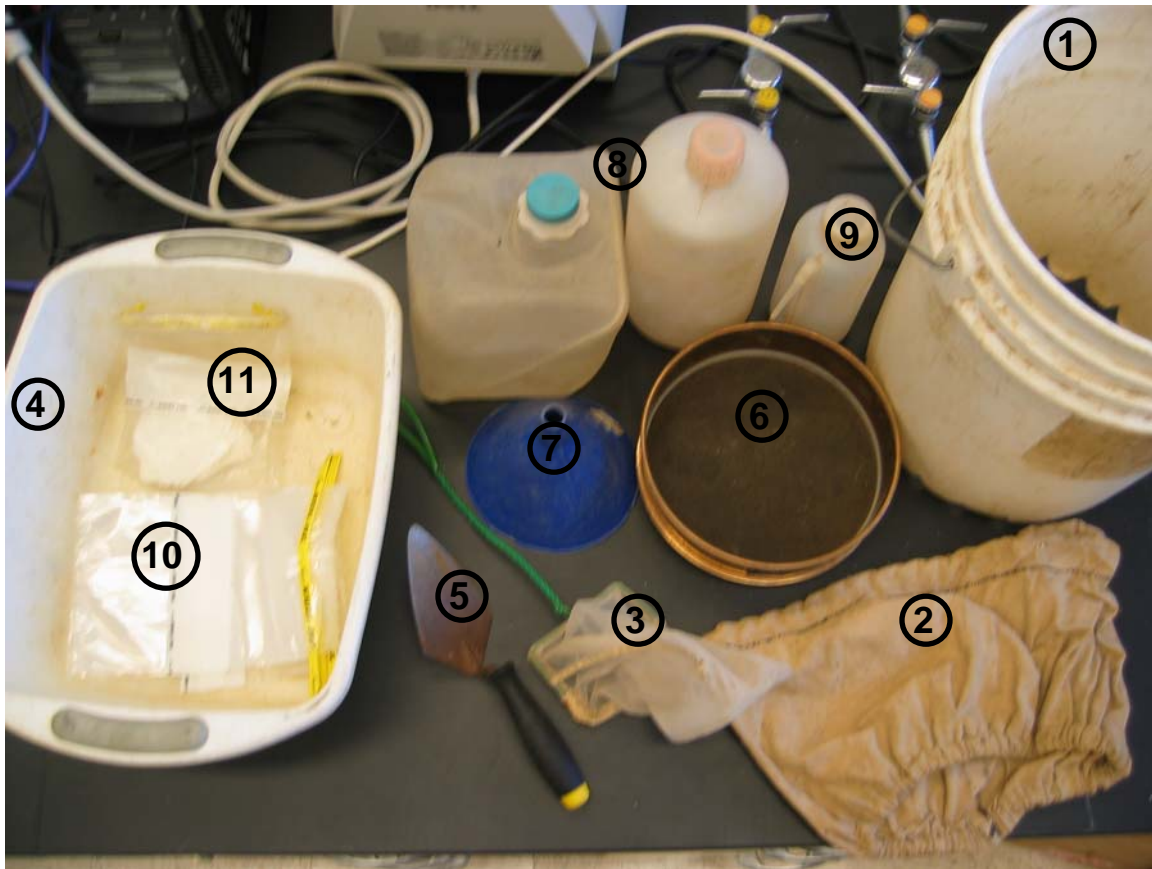


Figure 4-22 Equipment used to collect and preserve benthic invertebrate samples. Numbers correspond to the equipment and supplies list above.

4.5 Surveying the amphibian assemblage ***General***

This subsection provides instructions for characterizing amphibian assemblages in headwater streams using a visual encounter survey. All amphibians are highly dependent on water and the amount of moisture in the environment influences their geographic range, life history characteristics, and behavior. With the exception of the tailed frog in the western United States, most headwater stream-dwelling amphibians are urodels (salamanders) rather than anurans (frogs and toads). This discussion therefore focuses on the use of salamanders as

indicators of hydroperiod, but the methods are similarly effective for anurans populations.

Following hatching, all stream salamanders go through a gilled larval stage during which they are obligate to the aquatic environment. The larval stage may last from months to several years depending on species and locality. At the end of the larval stage, most species metamorphose into juveniles and leave the stream to become semi-aquatic or terrestrial as adults. Adults subsequently return to streams for courtship and egg-laying. Some salamanders are permanently aquatic as adults (eg., *Cryptobranchus alleganiensis*, *Necturus* spp., etc.) and retain their gills. All stream

salamanders are predatory and they are often the top predators in high-gradient, fishless headwaters (Davic and Welch 2004, Johnson and Wallace 2005).

The fact that stream-dwelling salamander larvae are obligate to the aquatic environment and have larval periods that can vary greatly in length means they are potentially ideal indicators of stream hydroperiod. This protocol focuses on the larval stage because adult salamanders are less dependent on water and may move far from the stream channel. Unfortunately, identification of larval salamanders can be difficult and few good comprehensive larval keys are available. Field crews should therefore attempt to become familiar with the salamander species in their area prior to sampling. Larvae of the two-lined salamanders (*Eurycea bislineata*, *E. cirrigera*, and *E. wilderae*) are among the most commonly encountered salamanders in streams of the eastern United States. Larvae are dusky colored dorsally, have branched external gills, and have 6-9 pairs of light dorsolateral spots (Figure 4-23B). The Appalachians are home to the greatest salamander diversity, and larvae of *Desmognathus* spp., *Gyrinophilus* spp. (Figure 4-24), and *Pseudotriton* spp. are also frequently encountered in streams of this region. Amphibian diversity is lower in the western United States, but species often encountered in streams include the giant salamanders (*Dicamptodon* spp.) and tailed frogs (*Ascaphus* spp.). Petranka (1998) provides a larval key and distribution map for salamanders of the United States. Other regional keys and distribution maps may be available for your area (e.g., Green and Pauley 1987, Pfingsten and Downs 1989, Minton 2001) and many larval descriptions can be found in the primary literature.

4.5.1 Time-constrained sampling

Time-constrained sampling is an effective way of sampling salamander larvae from a variety of habitats, typically with minimal cost, effort, and stream disturbance. While the timed-search method makes density determinations difficult, it can be used to estimate relative abundance of species. Timed sampling has an additional advantage in that it increases the chance of collecting rare taxa, or rare individuals when salamanders are scarce (Crump and Scott 1994, Barr and Babbitt 2001). Chalmers and Droege (2002) additionally found that timed-search sampling was more effective than use of leaf litter bags in estimating abundance of larval *E. bislineata*. We chose the time-constrained approach for this protocol because it is robust and best suited for collection of larvae from streams across multiple regions where species composition and densities may be highly variable. The method is also effective over a wide range of stream size, hydroperiod, and condition.

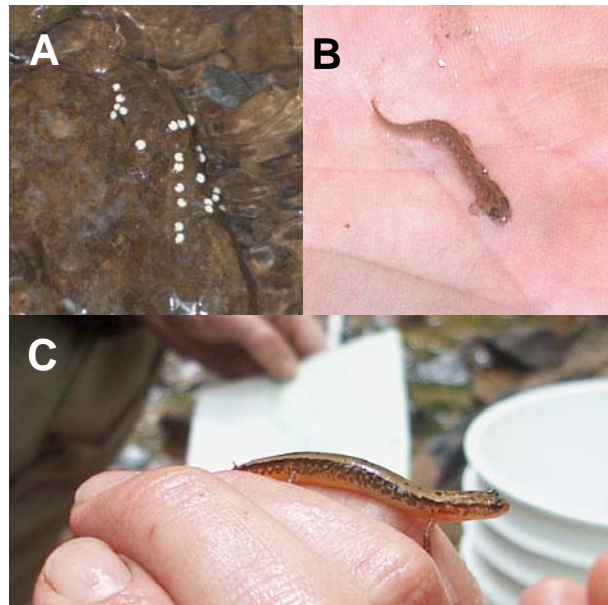


Figure 4-23 Northern two-lined salamander, *Eurycea cirrigera*, from Robinson Forest, KY: A) egg clutch; B) larva; and C) adult.



Figure 4-24 Larval spring salamander, *Gyrinophilus porphyriticus*, from Robinson Forest, KY.

Procedure

When possible, sampling should be conducted with clear skies to maximize visibility. The amphibian survey reach and primary sampling reach should have similar discharge and habitat characteristics. Sampling typically begins ca. 10-m upstream of the primary 30-m sampling reach and progresses in the upstream direction. If there is an obvious change in habitat or discharge (i.e., a tributary confluence or headcut) above the primary reach, then sampling should be conducted downstream of the primary sampling reach. Sampling begins after noting the starting time and then continues for exactly 30 minutes. Only one person should conduct the sampling to standardize the level of effort and, if possible, the same person should conduct the survey at each site to minimize sampling variability.

Sampling is confined to the wetted area of the stream because the survey focuses on the larval stage. One crew member moves carefully upstream (or downstream in some cases) turning loose cover objects (leaves,

cobble, woody debris, etc.) in all available habitat types (shallow, deep, fast, slow, etc.). Salamander larvae are often found in isolated backchannels or at the stream margins where there may only be a thin surface film. Cover objects are turned individually by hand rather than using kick nets or other more destructive sampling methods. This saves time in sorting through debris and allows for the survey to cover a greater stream area. The investigator should slowly and methodically turn random cover objects from bank to bank while moving along the stream. Salamanders encountered are carefully collected into the hand net for identification. It should not be the intent of the investigator to turn over every object in the stream and there is no distance objective. Surveys in larger perennial streams with a greater wetted area will therefore cover less stream length than surveys done in small intermittent stream reaches. The objective is to keep the level of effort the same in every study reach, regardless of stream size or habitat types. Approximate length (m) of stream surveyed should be noted at the end of the 30-minute survey.

Larval salamanders observed during the survey are identified in the field when possible and recorded on the amphibian survey field sheet along with corresponding life stage (larva, juvenile, adult) (Figure 4-25). Mean snout-vent length (SVL) for each cohort should be visually estimated (mm) for each species and recorded on the field form to help determine when larvae may have hatched. Presence of larger/older larvae of species with multi-year larval stages (e.g., *Desmognathus quadramaculatus*, *Gyrinophilus porphyriticus*, northern populations of *E. bislineata*) should be noted as this can be an important indicator of stream permanence. Unknown species and voucher specimens should be recorded photographically, showing top and side views at a minimum. Each species should be

vouchered for each area sampled (i.e., ecoregion, national forest, etc.). Salamanders should not be collected and returned to the laboratory without appropriate collection permits. Salamanders collected for vouchers or for species confirmation should be anaesthetized with 0.1% MS-222 (tricaine methanesulfonate) (Beachy 1994) and then preserved with a 10% formalin solution. Preserved specimens should be placed in vials labeled with the site name, date, and name of the collector. Photos and preserved specimens may be sent to regional experts for species confirmation. Though the survey is aimed at the larval stage, adults and egg clutches found during the survey should also be noted on the field form and photographed. Any fish observed during sampling should also be recorded.

4.5.2 Alternative methods

Salamanders have been collected from streams using a wide variety of sampling methods (reviewed by Heyer et al. 1994). Larvae may be qualitatively sampled using kick-nets or conventional dredge nets typically used in benthic macroinvertebrate sampling. Such collection methods; however, can be destructive and time-consuming and may not adequately represent rare taxa. Typical quantitative approaches include: other benthic sampling devices (e.g., benthic corers, Surber samplers), quadrats (e.g., Welsh and Lind 1996, Rocco and Brooks 2000), transects (e.g., Resetarits 1997, Welsh and Oliver 1998), and artificial habitats (Pauley 1998). Bury and Corn (1991) provide an example of a more intensive sampling method for western streams, whereby all moveable objects are removed from a 10-m stream section by a two people over ca. 5 hours.

AMPHIBIAN ASSESSMENT FORM					Page 4 of 4
SITE NAME: _____					DATE: / /
COLLECTION TIME: _____ to _____					REACH LENGTH COVERED (m): _____
SURVEYOR: _____					
SPECIES / LIFE STAGE					
SPECIES	#LARVAE	#JUVENILE	#ADULT	TOTAL	VOUCHER?
<i>Desmognathus fuscus</i>					
<i>Desmognathus monticola</i>					
<i>Desmognathus ochrophaeus</i>					
<i>Desmognathus welteri</i>					
<i>Eurycea bislineata</i>					
<i>Eurycea longicauda</i>					
<i>Gyrinophilus porphyriticus</i>					
<i>Pseudotriton montanus</i>					
<i>Pseudotriton ruber</i>					
OTHER					
NOTES ON AMPHIBIANS					
NOTES ON FISH (include species present)					

Figure 4-25 Amphibian survey field form.

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Equipment and supplies

Aquarium dip net (approximately 15.5 x 12
cm, 1-mm mesh)

Wristwatch or stopwatch

Specimen containers (optional)

0.1% MS-222 (optional)

10% formalin (optional)

Digital camera

Field forms